Degradation and detoxification of leather tannery effluent by a newly developed bacterial consortium GS-TE1310 for environmental safety

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Abstract

The untreated/partially treated effluent discharged from leather tanning industries is heavily polluting our water and soil resources. Hence, the adequate treatment/detoxification of tannery effluent (TE) is required before its safe disposal into the environment. In the present study, an effective degradation of real TE was attained by a newly developed bacterial consortium GS-TE1310 within 120 h with 76.12, 85.32, 71.89, 48.59, 78.81, 69.53, 71.22, and 88.70 % reduction in pollution parameters such as COD, BOD, TDS, phosphate, sulphate, nitrate, Cr, and phenol, respectively. The HP-LC, FT-IR, and GC–MS study showed that most of the organic contaminants identified in the untreated TE were completely mineralized/degraded into new degradation products in the treated TE by the newly developed bacterial consortium GS-TE1310 at 7 pH, 0.5 % glucose and ammonium chloride, 120 rpm, and 20 mL inoculum volume. Further, the bacterially treated TE was used for the phytotoxicity evaluation using *Phaseolus aureus* L as a terrestrial model organism. Results revealed that the toxicity of bacterially treated TE was significantly reduced, allowing the 70 % germination of seeds, and thus, confirmed the detoxification of leather TE. Overall, the newly developed bacterial consortium GS-TE1310 demonstrated a remarkable potential to efficiently treat/detoxify leather TE for environmental safety.

Keywords

Tannery effluent, Bacterial consortium, Bioremediation, GC–MS analysis, FT-IR analysis, Phytotoxicity

1. Introduction

Leather tanneries (LTs) hold a prominent place in the Indian economy and are technically known to process rawhide/skins for leather production. LTs are the key economic drivers of many developing nations

that considerably gain foreign exchange by exporting leather to other countries and create job opportunities for economically-deprived sections. Leather is the most important commodity marketed globally and almost 22,700.5 M ft^2 of leather is being produced worldwide every year [1] with a projected international market price of around 100 billion USD/year [2]. Nonetheless, LTs are also viewed as the most pollution creating industries as these releases a high volume of potentially toxic and hazardous effluent; however, its adequate treatment and management is a challenging task worldwide. Approximately, 30− 35 m^3 of effluent is being discharged during the processing of one ton of rawhide/skins in LTs [3]. Tannery effluent (TE) released from LTs invariably have obnoxious smell and dark brown colour with high pollution parameters such as biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), phosphate, nitrate, sulphate, phenol and a blend of noxious organic and heavy metal contaminants [4]. During leather processing, a huge quantity of noxious chemical compounds such as chromium (Cr), vegetable tannins, syntans, phenolics, azodyes, pesticides, sulphonated oils, polychlorinated biphenyls (PCBs), nonylphenols (NP), phthalates, etc., are currently applied to transform the rawhide/skins into leather/leather products [5]. These noxious chemical compounds are not completely uptaken by the rawhide/skins and consequently, discharged in the TE, which causes serious environmental threats and severe toxic hazards.

The untreated/partially treated TE discharged from LTs causes pollution of our natural resources (soil and water). In an aquatic ecosystem, TE reduces photosynthetic activity and oxygenation (depletion in dissolved oxygen) by blocking sunlight penetration in water bodies due to its dark brown color, and therefore, adversely affects flora and fauna [6]. In developing countries, TE is also being used as a liquid fertilizer by the local farmers to irrigate their food crops in the agricultural and (soil pollution). This uncontrolled and illegal practice causes the accumulation of deadly harmful metal toxicants like Cr at sequentially higher trophic-levels in the food chain (bioaccumulation) through eating/grazing by humans/animals and thus, creates severe health threats [7]. The toxic hazards of TE in both the aquatic and terrestrial ecosystem have been well-documented [8–14]. Thus, there is an urgent need to adequately treat/detoxify TE before its discharge to combat the environmental and health hazards.

Currently, the traditional activated-sludge treatment process (ASTP) applied at the common effluent treatment plants (CETPs) to treat/ detoxify TE is not feasible due to excessive sludge production and less removal efficiency for colour and refractory chemicals [7]. Several physico-chemical treatment technologies (such as coagulation/flocculation, adsorption, microfiltration, sedimentation, ozonation, photocatalysis, etc.) have been also developed by the time to improve the quality of treated TE. However, these treatment technologies are of limited scope because of secondary pollution problems issues and high operational and treatment costs and thus, are environmentally and economically not suitable for the effluent treatment [3,6]. Emerging Effluent Treatment Approaches such as anaerobic ammonium oxidation (ANAMMOX), membrane bioreactors (MBRs), membrane filtration technologies, and advanced oxidation technologies (electrochemical treatment, Fenton, etc.) are also available for the treatment of TE. But, the applications of emerging effluent treatment approaches at large scale are also uneconomical due to their energy-intensive nature and high operation costs and some other serious drawbacks [6] and thus, are less preferred in developing countries. Furthermore, to improve the pollutants removal from TE, various combined treatment technologies (biological treatment followed by physico-chemical treatment) has been also

developed [6]. However, these require major changes to the existing wastewater treatment plants (WWTPs) available with LTs. Therefore, an environmentally and economically feasible effluent treatment option with high treatment efficiency will be more preferable and acceptable to LTs.

Bioremediation technology (BT) has been increasingly recognized as an ecofriendly, safe, and cost-effective solution to effectively treat/ detoxify industrial effluents. BT employs microbes or plants to degrade/ detoxify the noxious contaminants in the industrial effluents. Several biological agents including bacteria [15], fungi [16], yeast [17], algae [18], and plants [19] have been studied for the degradation and detoxification of TE. In past, most of the researches were targeted to remove specific pollutants like Cr [4], phenol [20], and pentachlorophenol (PCP) [21] from TE. Moreover, TE is highly complex in nature because it contains a blend of organic and inorganic contaminants and hence, a monoculture of any biological agents could not efficiently treat/detoxify it. Conversely, the application of microbial consortia is more suitable over pure cultures to efficiently degrade/detoxify industrial effluents due to intensive metabolic activities of microbes equipped with broad enzymatic capacities that can effectively degrade a mixture of organic and inorganic contaminants [22].

To date, there is very limited detail available showcasing the use of bacterial consortia to degrade/detoxify real TE and some authors have used undefined microbial consortia [23,24]. Therefore, this study deals with the development of a new bacterial consortium GS-TE1310 using identified potential bacterial strains for the bioremediation of real TE. Besides, the environmental and nutritional factors were also optimized to enhance the pollutants removal efficiency in the real TE. Further, the prime objective of bioremediation is to lessen the toxicity of industrial effluents and hence, the phytotoxicity of TE before and after consortial treatment was also assessed to evaluate the environmental safety. This study is perhaps the first attempt on the development of a new bacterial consortium with identified potential bacterial strains and its application in the bioremediation and toxicity reduction in the TE after the secondary treatment. This study would be useful to develop a bacteria-based bioremediation process for WWTPs treating TE for environmental protection.

2. Materials and methods

2.1. Chemicals and media's

All the chemicals including reagents/solvents utilized in this study are of analytical grade with highest purity (purity ≥ 99 %) and bought from Sigma-Aldrich (St. Louis, MO, USA) whereas microbiological media were bought from HiMedia Laboratories (Mumbai, MH, IN). Mineral salt medium (MSM = K_2HPO_4: 2.0; Na_2HPO_4: 2.4; NH_4NO_3: 0.1; $CaCl_2$: 0.01; $MgSO_4$: 0.01 in. g/L) was used to isolate the potential bacterial strains for bioremediation of TE. Nutrient agar medium (NAM = yeast extract: 2; meat extract: 1.0; NaCl: 5.0; peptone: 5.0; agar: 15.0 in. g/L) was used to screen the potential bacterial strains. Whatman® glass microfibers filter papers (GF/C grade, 1.2 μm pore size; Whatman, England, UK) were utilized for the filtration of TE. The healthy seeds of mung-bean (*Phaseolus aureus* L.) were procured from a local seed shop of Lucknow (UP, IN) and utilized to study the phytotoxic effects of TE.

2.2. Collection of tannery effluent sample

TE prior and post biological (secondary) treatment was collected in a clean and sterilized plastic carboy (20 L capacity, Tarson Products Pvt. Ltd., Kolkata, WB, IN) from a discharge point of an aerobic activated-sludge (AAS)-based CETP of LTs situated in Unnao district (26.48 °N, 80.43 °E), UP, India. Further, the collected TE sample was instantly brought to the laboratory and stored at low temperature (4 °C) till the characterization of physico-chemical parameters, bacterial isolation (within 24 h), bioremediation studies and phytotoxicity assessment carried out.

2.3. Isolation of bacterial strains and culture conditions

For the isolation of bacterial strains to treat TE, the MSM broth [100 mL + 1% glucose (w/v) as carbon source, pH 7.0] was prepared using double distilled water in an Erlenmeyer flask (250 mL), sterilized (121 °C for 15 min) in the autoclave (SM-102, S M Scientific Instruments Pvt. Ltd., UP, IN), and then, kept for cooling at room temperature. 20 mL of real TE and 1 g of tannery sludge were added to the medium and incubated for bacterial enrichment at 35 °C and 120 rpm in incubator shaker (New Brunswick Innova 4230, NJ, USA) for five successive days. Afterward, the developed bacterial suspension (1 mL) was diluted serially and spread (50 μL) on the MSM agar plates, which were incubated for 24− 48 h in the temperature-controlled incubator shaker fitted with tray for the development of bacterial colonies. Further, the morphologically distinct bacterial colonies (GS1-GS10) were selected and picked up for purification by repeated streaking for the further screening.

2.4. Screening of potential bacterial strains

For better effluent treatability, the purified bacterial isolates were primarily screened on the basis of salt tolerance and selected as per their ability to tolerate high salt concentration and time required for adaptation as suggested by Sivaprakasam et al. [15]. The bacterial strains that showed tolerance to the high salt concentration were further selected for the secondary screening based on COD removal efficiency. For this, a loopful culture of the selected purified bacterial strains was cultured in MSM broth (50 mL, pH 7.0) supplied with 0.5 % glucose (w/v) as a C-source. 20 mL of overnight grown bacterial precultures were inoculated in TE (undiluted, 80 mL, pH 7.0) in the Erlenmeyer flasks (250 mL) which were kept for incubation at 35 °C and 120 rpm (shaking speed) in the incubator shaker. The bacterially treated TE sample was taken out every 24 h for successive five days, centrifuged (10,000 × g for 10 min) in a refrigerated centrifuge (REMI Instruments Pvt. Ltd., Mumbai, MH, IN) and the obtained supernatant was used to measure the COD of effluent. The bacterial strains that showed maximum COD removal in TE samples were selected for further studies. All the bacterial cultures were maintained on MSM agar plates (supplied with glucose, 1%, w/v) at 35 °C.

2.5. Identification of potential bacterial strains

Based on the results of salt tolerance and COD removal efficiency, three potential bacterial strains (GS1, GS3,and GS10) were selected and identified by morphological and biochemical characterization in line with procedures defined in the “Bergey’s Manual of Determinative Bacteriology [25]. The identity of the isolated

bacterial strains was also revealed by sequencing of 16S rRNA gene. For this, the genomic DNA was extracted and prepared for molecular characterization in line with procedure stated earlier [26]. The universal primers [27 F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′)] were used to amplify PCR products in

Veriti™ 96-Well Thermal Cycler (Applied Biosystems™ Inc., CA, USA) using 35 PCR thermocycles [first denaturation (for 2 min at 95 °C) followed by second denaturation (for 30 s at 95 °C) with annealing (for 30 s at 52 °C), extension (for 2 min at 72 °C), and terminal extension (for 15 min at 72 °C)]. The PCR product was purified using DNA Extraction Kit (Merck Life Science Pvt. Ltd., Bengaluru, KA, IN) and thereafter, sequenced on the ABI 3500 Genetic Analyzer using Big Dye Terminator software (v3.1). The nucleotide sequences of 16S rRNA gene were compared with available sequences using NCBI-BLAST and submitted to GeneBank to receive the accession numbers. Mega software (v7.0, www.megasoftware.net) was used to construct evolutionary tree using neighbour-joining method.

2.6. Development and performance evaluation of bacterial consortium GS-TE1310

Based on the performance of mono-cultures in the bioremediation studies for TE, a new bacterial consortium GS-TE1310 comprising potential bacterial strains GS1, GS3 and GS10 was developed after bio-interaction studies. For this, a loopful culture of the purified bacterial strains was aseptically transferred in the Erlenmeyer flasks (150 mL) containing 50 mL MSM broth (pH 7.0) supplied with 0.5 % glucose (w/ v) as a C-source and kept for incubation at 35 °C and 120 rpm (shaking speed) in the incubator shaker for 24 h. Further, 5 mL of each bacterial pre-culture was inoculated in MSM broth (85 mL, pH 7.0) amended with glucose (1%, w/v) as a carbon source in an Erlenmeyer flask (250 mL) and kept to incubate at 30 °C and 120 rpm in the incubator shaker for 24 h. Afterward, 20 mL of the newly developed bacterial consortium GS-TE1310 was inoculated in TE (undiluted, 80 mL, pH 7.0) in the Erlenmeyer flasks (250 mL) and kept for incubation (35 °C & 120 rpm) in the incubator shaker for successive five days to evaluate the performance in the bioremediation of TE. Further, the bacterially treated TE sample was taken out every 24 h, centrifuged (10,000 × g for 10 min) in a refrigerated centrifuge and the supernatant was used for the COD measurements.

2.7. Optimization of bacterial consortium GS-TE1310 at different parameters for maximum removal efficiency

To enhance the pollutant (COD) removal efficiency, a newly developed bacterial consortium GS-TE1310 was optimized for the nutritional and environmental parameters, inoculum concentration and shaking speed for the efficient bioremediation of TE. For the optimization of nutritional parameters, 20 mL of the developed bacterial consortium GS- TE1310 was inoculated in TE (undiluted, 80 mL, pH 7.0) supplied with different C-sources (sucrose, glucose, maltose, lactose, and starch; 0.5 %, w/v) and N-sources (urea, ammonium chloride, yeast extract, peptone, and sodium nitrate; 0.5 %, w/v) in the Erlenmeyer flasks (250 mL) and kept for incubation (at 35 °C) with parallel abiotic controls under shaking condition (120 rpm) in the incubator shaker. For the optimization of environmental parameters, 20 mL of the developed bacterial consortium GS-TE1310 was inoculated in TE (undiluted, 80 mL) in the Erlenmeyer flasks (250 mL). To this,

the optimized concentrations of C and N-source (0.5 %, w/v) was added, well-shaked and kept for incubation at a wide range of pH (5–9) at 35 °C and a wide range of temperature (25, 30, 35, 45 and 45 °C) with the optimized pH with parallel abiotic controls under shaking condition (120 rpm) in the incubator shaker. For the optimization of inoculum volume and shaking speed, different concentrations (4, 8, 12, 16, and 20 %, v/v) of the developed bacterial consortium GS-TE1310 was inoculated in the undiluted TE amended with the optimized nutrient (C & N source) concentrations (0.5 %, w/v) in the Erlenmeyer flasks (250 mL). Afterward, the flasks were kept with parallel abiotic controls for incubation at the optimized pH and temperature (°C) under different shaking conditions (100− 140 rpm) in the incubator shaker. All the experiments were done for successive five days. Afterward, the bacterially treated TE sample was taken out every 24 h, centrifuged (10,000 × *g* for 10 min) in a refrigerated centrifuge and the obtained supernatant was utilized in the COD measurements.

2.8. Bioremediation experiment

For the bioremediation studies, real undiluted TE amended with the optimized nutrient (C & N source) concentrations (0.5 %, w/v) was inoculated with the optimized inoculum concentration (%, v/v) of the developed bacterial consortium GS-TE1310 in the Erlenmeyer flasks (250 mL). Further, the flasks were kept for incubation with parallel abiotic controls at optimized environmental (pH and temp. (°C)) and shaking (rpm) conditions in the incubator shaker for successive five days. During the experiments, bacterial growth was observed by taking the absorbance (at λ_{max} =620 nm) using a spectrophotometer (Thermo Scientific™ Evolution 201, Australia). Afterward, the bacterially treated TE sample was taken out every 24 h, centrifuged (10,000 × g for 10 min) in a refrigerated centrifuge and the obtained supernatant was utilized in COD measurements. The open reflux method (Method No. 5220B) was used to measure the COD of TE and the calculation of removal efficiency was done as per the following formula:

$$\text{Removal Efficiency (\%)} = C_0\text{-}C_t/C_0 \times 100 \quad (1)$$

[Where C_0 = initial concentration of pollutant (mgL^{-1}) in the untreated TE; C_t = final concentration of pollutants (mgL^{-1}) in the TE after biotreatment].

2.9. Analytical methods

2.9.1. Physico-chemical characterization of tannery effluent

The physico-chemical characteristics (pH, COD, BOD, TDS, TSS, total nitrogen, phenol, phosphate, nitrate, and sulphate) of the filtered TE (both untreated and after treatment with the bacterial consortium GS-TE1310) were examined in triplicates to know the strength of pollution in line with procedures defined in the “Standard Methods for Examination of Water and Wastewater” [27].

2.9.2. Analysis of heavy metals in tannery effluent

The analysis of heavy metals (HMs) in the TE prior and post treatment with the newly developed bacterial consortium GS-TE1310 was done by acid digestion method [HNO_3 + $HClO_4$; 10 mL (6:1 proportion) at 85 °C]

(EPA Method No.: 3005A) in line with the "Standard Methods for Examination of Water and Wastewater" [27] and HMs were detected and quantified using inductively coupled plasma (ICP) spectrophotometer (Thermo Electron; Model IRIS Intrepid II XDL, USA).

2.10. Spectroscopic analysis

2.10.1. Fourier transform-Infrared (FT-IR) analysis

The functional groups of the toxic organic chemicals in TE before and after treatment with bacterial consortium GS-TE1310 was determined by FT-IR analysis. For this, the TE sample (10 mL, filtered) was dried in oven at 105 °C, mixed with FT-IR grade-KBr (in 1:30 proportion, purity ≥ 99 %), ground and fused to form a thin (13 mm diam. × 1 mm thk.) pellet underneath the vacuum utilizing PCI Analytics Cast Steel IR Hydraulic Press of 10 tons capacity and, and used to record the absorption spectrum using Nicolet™ 6700 FT-IR spectrometer (Thermo Fischer Scientific, MA, USA). The scanning was done in the mid-IR range (4000− 400 cm^{-1}, 4 cm^{-1} resolution) to record the spectra against a pure KBr background spectrum in the ambient air. The processing of spectral data was done using OMNIC™ software (v7.4) whereas the absorption peaks detected in the FT-IR spectrum were assigned as per standard reference manual "Introduction to Organic Spectroscopy" [28]. *2.11. Chromatographic analysis*

For chromatographic analysis, the TE sample before and after treatment with bacterial consortium GS-TE1310 was extracted by liquid/liquid extraction (LLE) procedure For this, the untreated and treated TE sample (50 mL) was centrifuged (5000 ×g, 4 °C, and 10 min) to remove the suspended solids/bacterial biomass and the obtained supernatant was treated with HCl (1 N) to acidify (pH ≤ 2.0) it and used for the thrice extraction with an equal amount of ethyl acetate (50 mL, HPLC & GC grade, ≥ 99.9 %) followed by dichloromethane (DCM, 50 mL, HPLC & GC grade, ≥ 99.9 %) in a separatory funnel (500 mL). Afterward, a solvent layer (of both the solvents) that used to contain ROPs was taken out in a beaker through filtration, mixed together, and kept at ≤ 40 °C for evaporation in the Rotary Evaporator (RE 120), Buchi, Flawil, Sweden) till the solvent is fully vaporized. Further, the obtained residues were cooled to dissolve in 3 mL of DCM, subject to filtration through syringe filter of 0.22 μm size (Millipore Ltd., Bedford, Ma, USA), and the resulted extract was utilized for the chromatographic (HP-LC/ GC–MS) characterization.

2.11.1. High performance-Liquid chromatography (HP-LC) analysis

The analysis of organic contaminants and their degradation products in the TE prior and post treatment with the newly developed bacterial consortium GS-TE1310 was done on 515 HP-LC system (Waters Corporation, Milford, MA, USA). The 515 HP-LC system was furnished with a 2487 absorbance UV/Vis detector via millennium™ software (v32), 1100 series diode array detector (Agilent Technologies, USA) and reverse phase C18 column (250 mm L × 4.6 mm ID, 5 μm PS) at 27 °C temperature with a mobile phase (1.0 mL min^{-1} flow rate) consisted of Milli-Q water plus acetonitrile (in 70:30 proportion, v/v). For this, 20 μL of the final extract was introduced into the HP-LC system through injection and analysed at 224 nm to monitor the biodegradation along with organic contaminants and their degradation products formed during bioremediation of TE.

2.11.2. Gas chromatography–mass spectroscopy (GC–MS) analysis

The characterization of organic pollutants and their degradation products in the TE prior and post treatment with the newly developed bacterial consortium GS-TE1310 was done on Thermo Scientific™ TRACE™ GC Ultra Gas Chromatograph System (Thermo Fischer Scientific, USA). The GC system was furnished including a Thermo™TriPlus autosampler combined to a Thermo™TSQ Quantum XLS™ Triple Stage Quadrupole Mass Spectrometer and DB-5 ms capillary column [30 m (L)

× 0.18 mm (ID) ×0.18 μm (FT), 5% (phenyl) + 95 % (methyl-

polysiloxane)] (Agilent Technologies, USA). Initially, the derivatization of the final extract was done with 2,2,2-Trifluoro-*N,*O-bis(trimethylsilyl) acetamide (TFBSA) and Trimethylsilyl Chloride (TMSCl) and thereafter, sample was dissolved in the running solvent (DCM) and 2 μL of it was introduced into the GC column through injection and analysed in full scanning mode at 3.0 min solvent delay. The recording of mass spectra (MS) was done at a standard energy of 70 eV and 1 mL min^{-1} flow rate of mobile phase (helium, purity ≥ 99.99 %). In the beginning, the temperature of column was fixed at 60 °C for 2 min and further raised to 290 °C for 20 min at a 10 °C increasing rate. Further, the MS was interpreted using NIST search databank (v1.0.0.12, NIST, USA) to ascertain the identity of organic contaminants and their degradation products by matching their MS with that of their retention times (RTs)

2.12. Phytotoxicity assessment

The phytotoxicity of TE before and after treatment with bacterial consortium GS-TE1310 was assessed using *Phaseolus aureus* L. (a terrestrial model used in toxicity evaluation) as per OECD Safety Guidelines (http://www.oecd.org/chemicalsafety/testing/33653757. pdf). A seed germination test was done in three replicates to evaluate the phytotoxicity of TE using healthy and surface-sterilized (with a solution of 2.0 % $HgCl_2$ to kill the seed-borne fungi succeeded by thrice washing with distilled water) seeds (10) of *Phaseolus aureus* L. For this, seeds were firstly placed on filter-paper discs (No. 52, Whatman England, UK) and treated with varying concentrations (25, 50, 75, and 100 %) of TE (10 mL, v/v) and tap-water as a control and kept at room temperature for six consecutive days to explore the concentration- dependent deleterious impact on the germination/growth of seed/ seedling. Further, the deleterious impact of TE on the activity of α-amylase was also ascertained in the irrigated seeds to evidently explore the phytotoxicity as per Bharagava and Chandra [29]. All the physiological parameters relevant to the germination/growth of seed/seedling were calculated as per Bharagava et al. [30].

2.13. Quality control and quality assurance

The each analytical batch of heavy metals (Cd, Pb, Fe, Zn, Cr, Mn, Cu, Ni, and As) was calibrated and their quality was assured using reference stock standards (Merck KGaA, Darmstadt, Germany) and blanks in three replicates to confirm the accuracy of the method.

2.14. Statistical analysis

Three replicates (n = 3) were set for all the laboratory experiments done to validate the results expressed as mean ± SD (standard deviation) values. All the calculations and statistical analysis were done on the IBM SPSS Statistics software (v20.0.0, SPSS Inc. Chicago, IL, US).

3. Results and discussion

3.1. Characteristics of bacterial strains

In this study, a total of ten (10) bacterial strains (GS1-10) were isolated from the TE + sludge sample collected from the discharge point of CETP, Unnao (UP) India. Further, the isolated bacterial strains (GS1-10) were subjected to the primary screening based on the salt (NaCl) tolerance index. In our study, out of ten (10) bacterial isolates (GS1-10), only seven (07) bacterial strains i.e. GS1, GS2, GS3, GS4, GS5, GS6, and GS10 were adapted to tolerate up to 6%, 4%, 4%, 3%, 4%, 3%,and 8% (w/v) salt (NaCl) concentration, respectively, over a wide range (1–10 %) of salinity. Further, these isolated bacterial strains (GS1-6 and GS10) were selected for the secondary screening based on COD removal efficiency. According to our study, only three bacterial strains i.e. GS1, GS3, and GS10 were reported to remove COD up to 61.12 %, 54.28 %, and 66.32 % in real TE within 120 h at 35 °C and 120 rpm. Moreover, these bacterial strains (GS1, GS3, and GS10) were also showed maximum tolerance to salt (NaCl) concentration up to 6%, 4%, and 8% (w/v) and thus, are halotolerant in nature and suitable to treat/detoxify TE.

Basis the results obtained for salt tolerance and COD removal efficiency, only three potential bacterial strains i.e. GS1, GS3, and GS10 were finally selected and identified based on various morphological and biochemical tests (done using HiMedia Kits). The colonies of bacterial strain GS1 and GS3 were looked as milky white and white in colour, respectively whereas bacterial strain GS10 appeared as greenish in colour on MSM agar plates (S1A, B, C of Supplementary file). The bacterial strain GS1 was appeared as gram-negative, motile and rod in shape. GS1 showed positive reactions for urease, lysine utilization, nitrate reduction, ornithine, glucose, adonitol and lactose whereas negative reactions for citrate utilization, phenylalanine deamination, sorbitol, H_2S production, and arabinose. The bacterial strain GS3 was appeared as gram-positive, non-motile and round in shape. GS3 showed positive reactions for malonate, citrate utilization, catalase, sucrose, and glucose whereas negative reactions for trehalose, Voges Proskauer's, ortho-nitrophenyl-β-galactoside, nitrate reduction, arginine, mannitol, and arabinose. The bacterial strain GS10 was looked as gram-negative, motile and rod in shape. GS10 showed positive reactions for urease, ornithine utilization, glucose, nitrate reduction, adonitol, arabinose, and lactose whereas negative reactions for lysine utilization, citrate utilization, phenylalanine deamination, H_2S production, and sorbitol. Based on the morphological and biochemical tests, the isolated bacterial strain GS1, GS3, and GS10 probably belonged to the *Ochrobactrum, Micrococcus* and *Stenotrophomonas* genera, respectively.

Further, on basis of 16S rRNA gene sequencing, the bacterial strains GS1, GS3, and GS10 were identified and confirmed as *Ochrobactrum intermedium* (MK344317), *Micrococcus lylae* (MK344318), and *Stenotrophomonas acidaminiphila* (MK344319). The sequence similarity index (SMI) was calculated using nucleotide sequences of the 16S rRNA genes of bacterial strain GS1, GS3, and GS10. The bacteria strain GS1,

GS3, and GS10 was homologous to *Ochrobactrum intermedium*, *Micrococcus lylae*, and *Stenotrophomonas acidaminiphila* 16S rRNA gene sequences retrieved from BLAST search and the SMI was 97–100 %. The evolutionary tree was constructed using neighbour-joining method (bootstrap consensus test) using MEGA software (v7.0) (S2A, B, C of Supplementary file). According to phylogenetic tree, the bacterial strains GS1, GS3, and GS10 was closely related to the *Ochrobactrum intermedium* (NR113812.1), *Micrococcus lylae* (NR026200.1), and *Stenotrophomonas acidaminiphila* (NR025104.1), respectively. Further, our findings are supported by previous studies that report the potential of *Ochrobactrum, Micrococcus,* and *Stenotrophomonas* genera in the biodegradation and bioremediation of environmental contaminants/industrial wastes [31–35].

3.2. Bioremediation of tannery effluent using bacterial consortium GS-TE1310

TE is oftenly a blend of potentially-toxic organic chemicals and heavy metals and thus, its treatment and detoxification are necessary for environmental protection. COD is considered as one of the most important pollution parameters and used as norm to measure the strength of pollution of waster/wastewaters [27]. Industrial effluents with exteremly high COD disturb the ecological functioning of aquatic ecosystem and thus, adversely affect flora and fauna [6]. Hence, the removal of COD from TE is utmost required to detoxify TE for the environmental and public health safety.

In the present study, the bioremediation of TE is primarily monitored in terms of reduction in COD according to Eq. 1. For this, batch studies (in shaking flasks) were performed for the bioremediation of TE with pure monocultures of three isolated potential bacterial strains, *Ochrobactrum intermedium* GS1, *Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10 and their consortium. During bioremediation studies, the COD removal from real TE was 61.12 %, 54.28 %, and 66.32 % by the *Ochrobactrum ientermedium* GS1, *Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10, respectively, within 120 h at 35 °C and 120 rpm. However, the COD removal from real TE by the newly developed bacterial consortium GS-TE1310 comprising *Ochrobactrum intermedium* GS1, *Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10 was much higher (74.15 %) as compared to the individual bacterial strains within 120 h at 35 °C and 120 rpm (Fig. 1). This faster COD removal from real TE by the newly developed bacterial consortium GS-TE1310 might be attributed to the diverse catabolic activity of consortium that might degraded a variety of contaminants present in the complex wastewater and thus, the degradation was higher [36]. Microbial consortia comprises potential bacterial strains and are considered as an effective tool for bioremediation and wastewater treatment because intermediate compounds of a catabolic pathway of one bacterial strain are further degraded via suitable catabolic pathway of other bacterial strains and thus, effectively treat/detoxify recalcitrant industrial effluents as compared to individual bacterial strains [22]. Further, a high COD removal is also confined to the state of the mineralization of pollutants [37].

To enhance the COD removal efficiency, the newly developed bacterial consortium GS-TE1310 was optimized for environmental (pH and temperature) and nutritional (carbons and nitrogen sources) parameters, and inoculum concentration and shaking speed for effective degradation of real TE. The results of the optimization of C-sources (0.5 %, w/v) shown that a maximum COD reduction (74.68 %) was noted in

presence of glucose as additional C-source within 120 h at 35 ˚C and 120 rpm, followed by lactose(68.48 %), maltose (56.32 %), sucrose (44.28 %), and starch (38.58 %) (Fig. 2A). Glucose was might be acted as an external co-substrate [38] that enhanced the degradation of TE by the newly developed bacterial consortium GS-TE1310. Contrarily, in case of N-sources (0.5 %, w/v), the maximum COD reduction (74.92 %) was recorded in presence of ammonium chloride (NH_4Cl) as a major source of nitrogen within 120 h at 35 ˚C and 120 rpm, followed by sodium nitrate (72.28 %), urea (68.52 %), yeast extract (61.39), and peptone (38.68 %), respectively (Fig. 2B).

Besides, the results of the pH optimization showed that the newly developed bacterial consortium GS-TE1310 was able to remove COD within a wide range of pH (5–9) (Fig. 2C). The maximum COD reduction (75.18 %) was recorded at pH 7, indicating that the neutral pH highly favored the bacterial growth and metabolism during the bioremediation of TE whereas, at pH 6, 8 and 9, the COD reduction was54.28 %, 68.72 %, and 56.12 %, respectively. However, the COD removal was least (42.14 %) at pH 5 because of acidic conditions. The common salt (NaCl) used in soaking operation imparts a neutral pH to effluent [15] and hence, this newly developed bacterial consortium GS-TE1310 is suitable for the treatment and detoxification of TE without any pH correction. Further, temperature optimization studies proved that the newly developed bacterial consortium GS-TE1310 was able to remove COD within a wide range of temperatures (25–45 ˚C). In the present study, the maximum COD reduction (75.52 %) was recorded at 35 ˚C while the least COD reduction (43.36 %) was noted at 25 ˚C (Fig. 2D). This was might be due to higher bacterial activity at 35 ˚C; however, CETPs in the

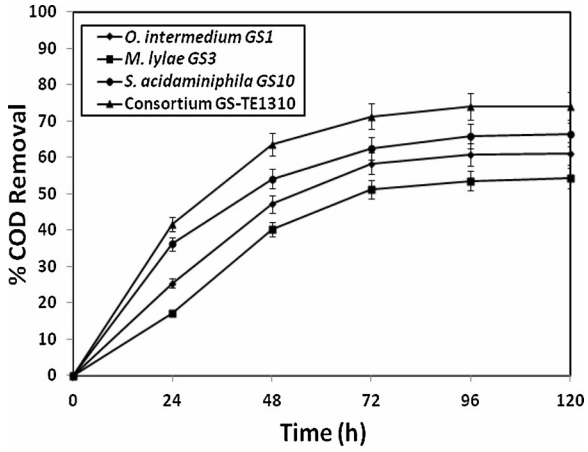


Fig. 1. COD removal from real tannery effluent by the newly developed bacterial consortium GS-TE1310 and individual bacteria, *O. intermedium*, *M. lylae*, and *S. acidaminiphila.* Error bars represent the standard deviation calculated from at least three independent experiments performed at standard conditions (7 pH, 35 ˚C, and 120 rpm).

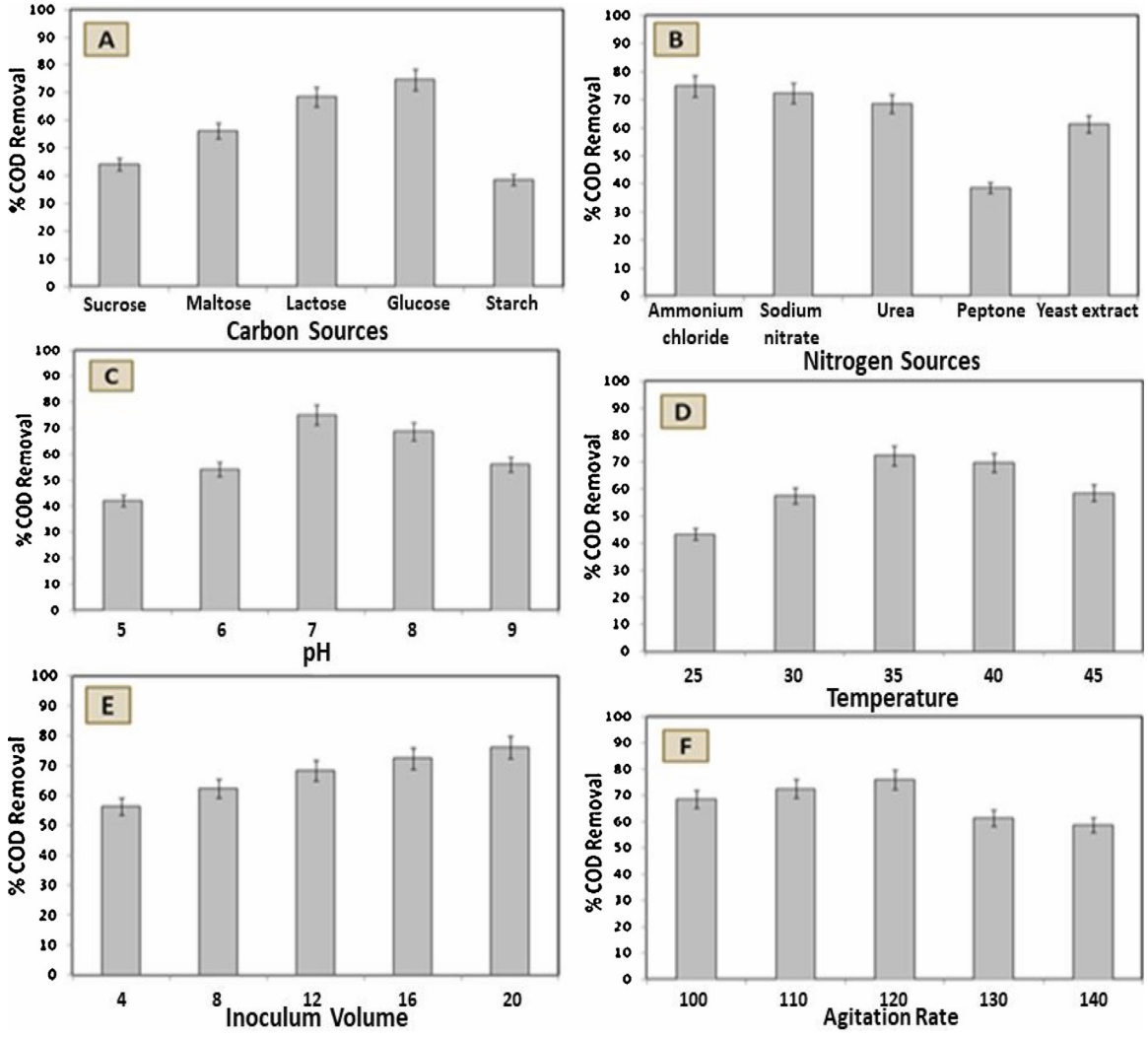


Fig. 2. Effect of (A) carbon sources (B) nitrogen sources (C) pH (D) temperature (E) inoculum concentration, and (F) agitation/shaking speed on COD removal from real TE using newly developed bacterial consortium GS-TE1310. Error bars represent the standard deviation calculated from at least three independent experiments.

tropical countries (like India) are mainly operated at normal day temperature (≥ 30 °C) [15] and thus, this bacterial consortium is suitable for the degradation of TE.

Further, the results of the inoculum concentration optimization showed that only 20 % inoculum concentration (v/v) was ascertained to be optimum inoculum volume for the maximum COD reduction (75.86 %) from real TE by the newly developed bacterial consortium GS- TE1310 (Fig. 2E). This was might be attributed to the higher cellular biomass production and thus, higher the metabolic activities of the bacterial strains capable to degrade/detoxify the contaminants present in the TE. Besides, the results of the shaking speed optimization showed that only 120 rpm was found to be optimum shaking speed for the maximum COD reduction (76.08 %) from real TE by the newly developed bacterial consortium GS-TE1310 within 120 h at 35 °C and 7 pH (Fig. 2F). This was probably due to proper mixing of oxygen that improved the bacterial cell density and biomass as well as oxygen transfer amid the medium and bacterial cells [38]. Further, an increase in shaking/agitation rate (130− 140 rpm) caused an observable fall in COD removal efficiency (17.30

%) and was might be due to shear rate effect on the bacterial cell wall, leading to cellular damage (mechanical injury to bacterial cells) [15]. After optimization, the bioremediation of real TE was done at the optimized conditions (0.5 % glucose and NH_4Cl (w/v), 7 pH, 35 °C, 120 rpm and 20 mL inoculum volume) to testify the degradation potential of the newly developed bacterial consortium GS-TE1310. Results confirmed that the newly developed bacterial consortium GS-TE1310 efficiently removed a maximum COD (76.12 %) from real TE within 120 h (Fig. 3) and thus, demonstrated tremendous potential to degrade the leather TE.

3.3. Characteristics of tannery effluent

The results of the physico-chemical characterization of TE prior and post treatment with the newly developed bacterial consortium GS- TE1310 are listed in Table 1. The analysis of untreated TE confirmed that it has extremely high COD (1428 ± 5.56 mg L^{-1}), BOD (436 ± 4.58 mg L^{-1}), TDS (4064 ± 3.46 mg L^{-1}), TSS (2216 ± 2.64 mg L^{-1}), phosphate (118.66 ± 5.03 mg L^{-1}), sulfate (6.75 ± 0.27 mg L^{-1}), nitrate (14.05 ± 0.16 mg L^{-1}) and phenol (8.68 ± 0.04 mg L^{-1}). Moreover, the secondary-treated TE found to have a obnoxious odour and dark brown color with alkaline pH (8.2). Further, heavy metals like Cd (1.18 ± 0.03), Pb (0.38 ± 0.03), and Cr (6.88 ± 0.02 mg L^{-1}) were also detected in high amount in the untreated TE. All the pollution parameters testified in this study were beyond the standard limits for effluent release (Table 1).

The obnoxious odour was atributed to the presence of sulfate in the untreated TE [30]. However, the dark brown color of TE was atributed to dye azo compounds used to color leather in LTs [30]. A high BOD is indicative of the poor dissolved oxygen in the effluent and it was might

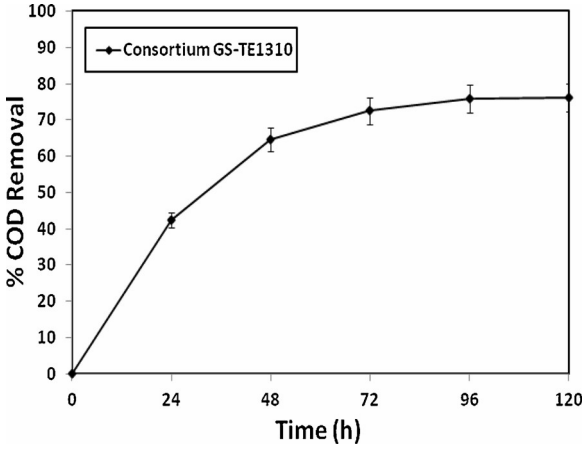


Fig. 3. Effect of inoculum concentration on COD removal from real TE using newly developed bacterial consortium GS-TE1310. Error bars represent the standard deviation calculated from at least three independent experiments (performed at the optimized conditions: 7 pH, 35 °C, 120 rpm, 20 mL inoculum volume, and 0.5 %, w/v glucose and NH_4Cl).

be atributed to a high organic matter present in the untreated TE [39]. A high COD and TDS were might be atributed to the recalcitrant organic pollutants, dissolved salts, and minerals present in the untreated TE [30, 36] and thus, the collected effluent is of very high pollution strength. A high TSS was might be atributed to the heavy metals present in the untreated TE [30]. Suspended solids are used to carry heavy metals and create toxicity in aquatic ecosysten when toxic metals get dissolved in water as a result of biochemical reactions [36]. A high content of nitrate, phosphate, and sulfate in the untreated TE were might be due to

the use of relevant chemical compounds/salts in the leather production processes. A huge amount of sulfuric acid/sulfide, mono or disodium phosphate/polyphosphate, and ammonium salts are commonly used in the dehariring, leather treatment, and deliming/bating, respectively, during leather processing [7]. A high content of phenol, Pb, Cd, and Cr were also recorded in the untreated TE and probably it was due the relevant chemical compounds used in leather treatment/processing in LTs. A high amount of PCP (C_6HCl_5O), NP ($C_{15}H_{24}O$), basic chomium sulfate [$Cr_2(SO_4)_3$], lead chromate ($PbCrO_4$as fatening agent), and Cd-based dyes/pigments are used in the preservation, finishing, tanning, marking/surfacing, and coloring of leather, respecticely [6].

The physico-chemical characterization of TE treated with the newly developed bacterial consortium GS-TE1310 showedan appreciable reduction in all the pollution parameters (Table 1). After bacterial treatment, the color of TE has turned from dark brown to light brown and this was might be due to the degradation and decolorization of the dyes present in the untreated TE [39]. The pH of TE was also significantly reduced from 8.2 ± 0.05 to 6.2 ± 0.11. The treatment of TE with the newly developed bacterial consortium GS-TE1310 resulted in the significant removal of BOD, TDS, TSS, phosphate, sulphate, nitrate, and phenol by 85.324 %, 71.89 %, 47.87 %, 48.59 %, 78.81 %, 69.53 %, 88.70 %, respectively after 120 h at 35 °C, 5 pH, and 120 shaking speed. The removal of BOD, TDS, TSS, phosphate, sulphate, nitrate, and phenol

from TE was might be attributed to the biodegradation/biotransformation of organic chemicals and heavy metals and utilization of dissolved minerals and salts to meet the nutritional requirements by the bacterial consortium GS-TE1310 [36,39]. Moreover, the HMs like Cr, Cd, Cu, Ni, and Fe were significantly removed by 71.22 %, 74.57 %, 85.46 %, 73.52 %, and 81.81 % from TE and their concentrations were found within permissible limits (Table 1). Besides, other HMs like Pb, As, Zn, and Mn were not detected in the TE after bacterial treatment. A significant reduction in the concentration of HMs was might be due to either the accumulation of metals inside the bacterial cells or their extracellular binding with lipopolysaccharides (LPS) of bacterial cell wall [35]. Overall, this newly developed bacterial consortium GS-TE1310 successfully reduced all the pollution parameters in TE during its bioremediation.

Table 1. Physico-chemical characteristics of tannery effluent.

Physico-chemical parameter	Effluent discharge limits*	UT-TE	BT-TE	Pollutant removal efficiency (%)**
Color	–	Dark brown	Light brown	–
Odour pH	–	Objectionable	Unobjectionable	–
Temperature (°C)	6.0– 9.0	8.2 ± 0.05	7.4 ± 0.11[b]	–
BOD_5 (mgL^{-1}) COD (mgL^{-1})	<35	32 ± 0.57	31 ± 1.15[ns] 64 ± 1.53[a]	–
	30.00	436 ± 4.58		85.32
TDS (mgL^{-1})	250.00	1428 ± 5.56	341 ± 2.64[a]	76.12
TSS (mgL^{-1})	2100.00	4064 ± 3.46	1142 ± 2.64[a] 1155 ± 2.88[a]	71.89
Phosphate (mgL^{-1})	100.00	2216 ± 2.64		47.87
Sulfate (mgL^{-1})	5.0	118.66 ± 5.03	61 ± 2.61[a]	48.59
Nitrate (mgL^{-1})	–	6.75 ± 0.27	1.43 ± 0.06[a]	78.81
Phenol (mgL^{-1})	10.0	14.05 ± 0.16	4.28 ± 0.03[a]	69.53 88.70
Heavy metals (mgL^{-1})	1.0	8.68 ± 0.04	0.98 ± 0.14[a]	71.22
Cr Cd Cu	2.0	6.88 ± 0.02	1.98 ± 0.12[a]	74.57
	0.05	1.18 ± 0.03	0.30 ± 0.06[a]	85.46
	3.0	1.72 ± 0.05	0.25 ± 0.02[a]	
Zn Ni	5.0 3.0	0.96 ± 0.03 0.68 ± 0.02	ND	–
			0.18 ± 0.02[a]	73.52
Pb	0.1	0.38 ± 0.03	ND	–
As Fe	0.2 3.0	BDL	ND	–
		2.86 ± 0.30	0.52 ± 0.05[a]	81.81
Mn	2.0	0.72 ± 0.04	ND	–

EC: Electrical conductivity; BOD: Biochemical oxygen demand; COD: Chemical oxygen demand; TS: total solids; TDS: total dissolved solids; TSS: Total suspended solids; BDL: Below detection limit; ND: Not detected; UT-TE: Untreated tannery effluent; BT-TE: Bacterially (consortial) treated tannery effluent.

All the values are mean of three replicates ± SD.

Data were analysed by Student's *t*-test [two tailed as compared to untreated sample].

[a] Highly significant at $p < 0.001$.

[b] Less significant at $p < 0.05$. [ns] Non significant at $p > 0.05$.

[*] As per Central Pollution Control Board (2010); Ministry of Environment, Forest & Climate Change (2016), India.

[**] Pollutant removal efficiencies (%) were calculated according to Eq. 1.

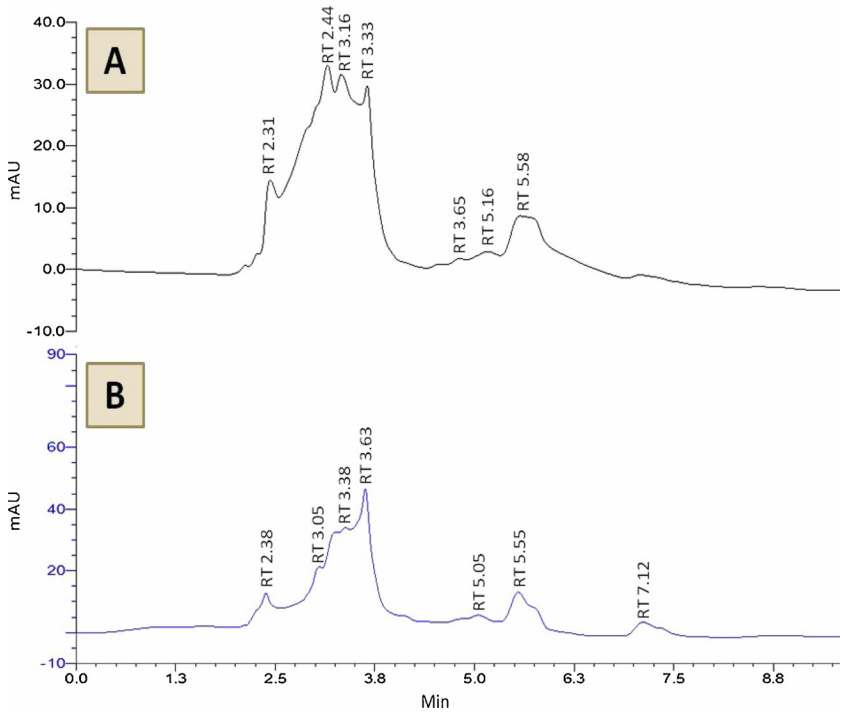


Fig. 4. HP-LC chromatogram of untreated tannery effluent (A) and treated tannery effluent (B) by the newly developed consortium GS-TE1310.

3.4. Characterization of recalcitrant organic pollutants and their degradation products

HP-LC, FT-IR, and GC–MS techniques were employed to characterize the organic contaminants/degradation products formed during bioremediation of real leather TE by the newly developed bacterial consortium GS-TE1310. According to HP-LC analysis, the untreated TE contained a mixture of organic contaminants as revealed by the several peaks obtained at different retention times (RT: 2.31, 2.44, 3.16, 3.33, 3.65, 5.16, and 5.58) (Fig. 4). However, the reduction in peak area (reduction in pollutants concentration) has clearly showed the biodegradation or biotransformation of organic contaminants and the newly formed metabolic products as confirmed by some additional peaks (at different RT: 2.38, 3.05, 3.38, 3.63, 5.05, 5.55, and 7.12) obtained in the bacterially treated TE, which were further confirmed using FT-IR and GC–MS techniques.

The FT-IR spectrum of untreated TE showed several peaks that correspond to toxic functional groups in organic contaminants present in the untreated TE. A high intensity absorption peak was observed in the range of 3694.7– 3423.5 cm^{-1} and assigned to O–H stretching indicated the phenol and alcohol derivatives whereas an absorption peak at wavenumber 2927.5 cm^{-1} denoted the C–H stretching suggestive of diazo and long-chain aliphatic compounds (fatty acids and surfactants) probably raised from phthalates and azo dyes applied in colouring/finishing of leather in LTs. An absorption peak at wave number 1637.1 cm^{-1} reflected the N–H bending ascribed to the amines and amides whereas CH_3 bending was observed at 1445.0 cm^{-1}. A C–N stretching signifying the aliphatic amines was recorded at wave number 1138.6 cm^{-1} whereas an absorption peak confined to the presence of P-O-alkyl organophosphorous compounds was assigned at wavenumber 995.5 cm^{-1}.Further, a absorption peak indicative of 1,2,4-trisubstituted benzene was also

observed at wave number 873.3 cm^{-1} and the O–C = O bending confined to carboxylic acids present in the sample was recorded at the wave number 616.7 cm^{-1}. To sum up, the results of the FT-IR analysis suggest the presence of alcohol, aliphatic amines, surfactants, phenols, azo dyes, carboxylic acids and aromatic skeleton in the untreated TE. However, the disintegration of some major peaks assigned to functional groups of toxic compounds and emergence of some new peaks suggested the degradation/transformation of organic contaminants in the bacterially treated TE (Fig. 5A & B).

Further, it is impossible to confirm the metabolism of a particular compound in the effluent containing "*n*" number of ROPs. The GC–MS characterization of untreated TE revealed the presence of a variety of organic contaminants (mostly are surfactants, endocrine disruptors, and aquatic toxicants) at different RT. However, most of the organic contaminants detected in the untreated TE were completely mineralized/ degraded into new metabolic products in the TE treated using the newly developed bacterial consortium GS-TE1310 at the optimized conditions (7 pH, 35 °C temperature, 0.5 % glucose and ammonium chloride (w/v), 120 rpm (agitation rate), and 20 mL inoculum volume)) (Fig. 6A & B; Table 2). The disappearance of most of the organic contaminants from untreated TE revealed that the newly developed bacterial consortium GS-TE1310 consumed these organic chemicals as a nutrient/energy source for the growth and development [39,36] and thus, effectively degraded/detoxified the leather TE.

3.5. Phytotoxicity of tannery effluent

The Ganga River and Unnao district near Kanpur (UP), India are highly polluted due to excess release of TE from LTs into the receiving environment (soil/water) that adversely affect the environment and create danger for nearby fauna and flora, and hence, this work is of prime importance in the current scenario. In the present study, the untreated TE was proved to be highly toxic and caused a reduction in the physiological parameters relevant to the germination/growth of seed/ seedling in *Phaseolus aureus* L, which were

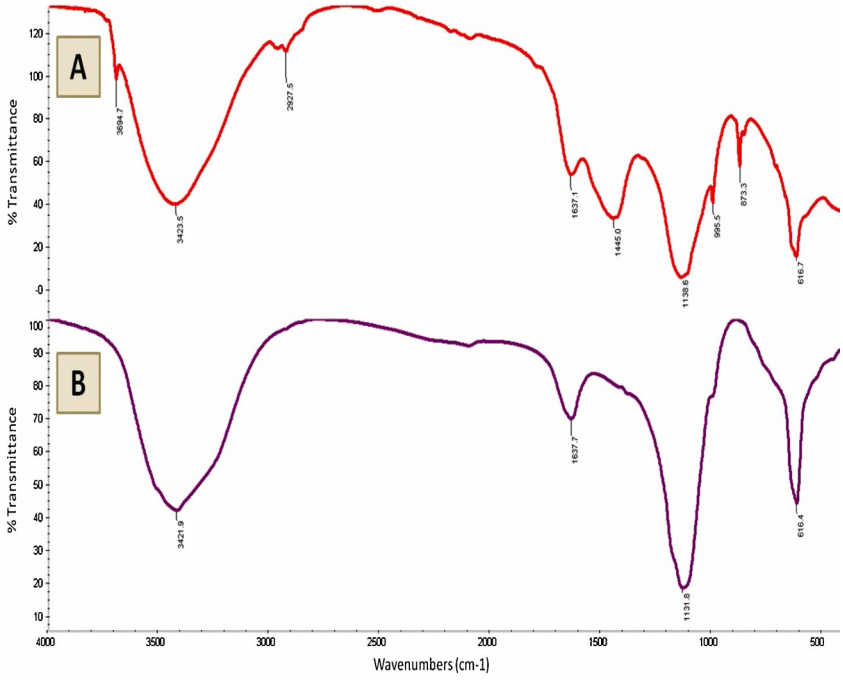


Fig. 5. FT-IR spectrum of untreated tannery effluent (A) and treated tannery effluent (B) by the newly developed consortium GS-TE1310.

significantly improved when seeds were irrigated with TE treated with the developed bacterial consortium GS-TE1310 (Table 3, Fig. 7A & B). This was perhaps due to bacterial degradation/detoxification of noxious organic chemicals and metals contaminants reported in TE as confirmed by physico-chemical characterization (Table 1) and FT-IR, HP-LC and GC–MS analysis. A very low germination percentage (GP) was observed in the seeds irrigated by varying concentrations (25, 50, 75, and 100 %, v/v) of untreated TE than control (TP: tap water). However, the GP was significantly improved in all the seeds treated with the different concentrations (25 %–100 %, v/v) of TE treated with bacterial consortium GS-TE1310. The phytotoxicity percentage (PP) was varied from 91.66 % - 47.72 % from higher to lower concentrations (100 % - 25 %, v/v) of untreated TE. This trend was perhaps due to a high salts load and toxic organic pollutants and metals present in the untreated TE that used to induce an anaerobic situation and high osmotic pressure, resulting in the uptake of toxic metals in plants and exert toxic effects [29]. Further, the PP was significantly improved when seeds were irrigated with the different concentrations (100 % - 25 %, v/v) of TE after treatment with bacterial consortium GS-TE1310 that degraded/detoxified the organic chemicals and toxic metals in the TE. However, the deleterious impact of industrial effluents on the physiological parameters relevant to the germination/growth of seed/seedling may vary according to its concentration and type of crops used for irrigation [30].

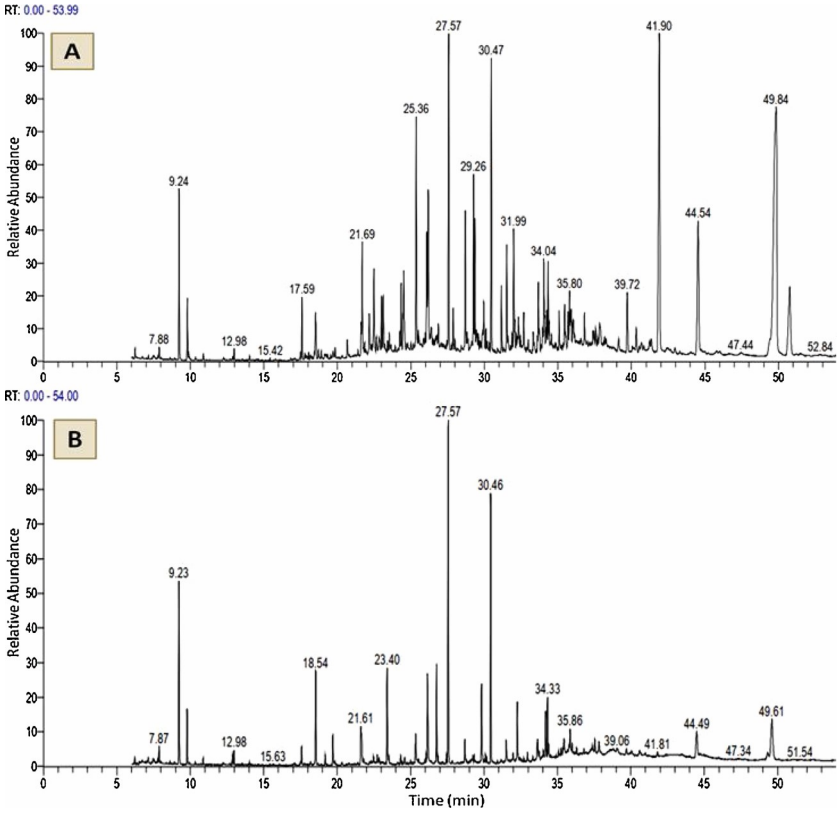
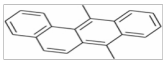
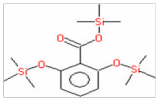
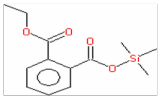
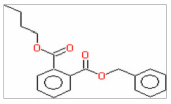
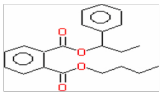
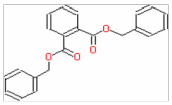
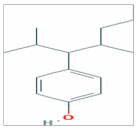
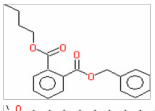
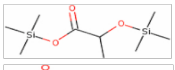
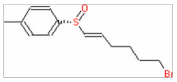


Fig. 6. GC–MS chromatogram of untreated tannery effluent (A) and treated tannery effluent (B) by the newly developed consortium GS-TE1310.

Table 2. Organic pollutants and their metabolic products identified as TMS (trimethylsilyl) derivatives by GC–MS analysis in untreated and treated tannery effluent by the newly developed bacterial consortium GS-TE1310.

Sr. No.	RT (min)	Compounds identified	Molecular formula	Molecular weight	Molecular structure	Tannery effluent	
						UT-TE	BT-TE
1.	7.88	Heptanoic acid, 7-phenoxy-, trimethyl ester	$C_{16}H_{26}O_3Si$	294		+	–
2.	9.24	Benz[a]anthracene,7,12-dimethyl-	$C_{20}H_{16}$	256		+	–
3.	12.98	Dodecane, 4,6-dimethyl-	$C_{14}H_{30}$	198		+	+
4.	15.42	Benzoic acid, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370		+	–
5.	17.59	Octadecane	$C_{18}H_{38}$	254		+	–
6.	21.69	p-Trimethylsiloxynitrobenzene	$C_9H_{13}NO_3Si$	211		+	–
7.	25.36	1,2,Benzenedicarboxylic acid, ethyl(trimethylsilyl)ester	$C_{13}H_{18}O_4Si$	238		+	–
8.	27.57	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	328		+	+
9.	29.26	Trans-9-Octadecanoic acid, trimethylsilyl ester	$C_{21}H_{42}O_2Si$	354		+	–
10.	30.47	Benzyl butyl phthalate, benzyl butyl ester	$C_{19}H_{20}O_4$	312		+	–
11.	31.99	3-Chloropropionic acid, heptadecyl ester	$C_3H_5ClO_2$	108		+	–
12.	34.04	17-Pentatriacontene	$C_{35}H_{70}$	490		+	–
13.	35.04	Phthalic acid, 1-phenylpropyl butyl ester	$C_{21}H_{24}O_4$	340		+	–
14.	35.80	Di-benzyl phthalate, dibenzyl ester	$C_{22}H_{18}O_4$	346		+	–
15.	39.72	4-(2,4-dimethylheptan-3-yl)phenol	$C_{15}H_{24}O$	220		+	–
16.	41.90	Tetradecanoic acid, trimethylsilyl ester	$C_{17}H_{36}O_2Si$	300		+	–
17.	44.54	Phthalic acid, benzyl isobutyl ester	$C_{19}H_{20}O_4$	312		+	–
18.	47.44	Stearic acid, 3-(octadecyloxy)propyl ester	$C_{39}H_{78}O_3$	594		+	–
19.	49.84	Dodecanoic acid, trimethyl ester	$C_{15}H_{32}O_2Si$	272		+	–
20.	52.84	Unknown compound	–	–	–	+	–
21.	7.87	D-(-)-Lactic acid, trimethylsilyl	$C_9H_{22}O_3Si_2$	234		–	+
22.	9.23	Pentanoic acid	$C_5H_{10}O_2$	102		–	+
23.	15.63	Unknown compound	–	–	–	–	+
24.	18.54	Unknown compound	–	–	–	–	+
25.	21.61	2-Methyl-1,2-propanediol 2TMS	$C_{10}H_{26}O_2Si_2$	234		–	+
26.	23.40	1-Octadecanol	$C_{18}H_{38}O$	270		–	+
27.	30.46	Octadecanoic acid, trimethylsilyl ester	$C_{21}H_{44}O_2Si$	356		–	+

(*continued on next page*)

Table 2 (*continued*)

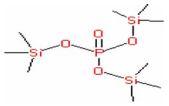
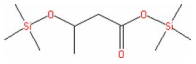
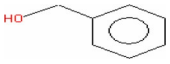
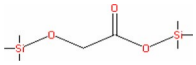
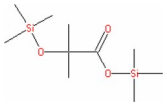
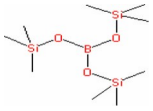
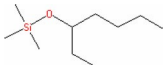
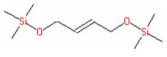
Sr. No.	RT (min)	Compounds identified	Molecular formula	Molecular weight	Molecular structure	Tannery effluent UT-	BT-
28.	34.33	Phosphoric acid, tris(trimethylsilyl) ester	$C_9H_{27}O_4PSi_3$	314			
29.	35.86	Butanoic acid, 3-[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{10}H_{24}O_3Si_2$	248		–	+
30.	39.06	Benzyl alcohol-MONOTMS	$C_{10}H_{16}OSi$	180		–	+
31.	41.81	Acetic acid, trimethyl silyl ester	$C_8H_{20}O_3Si_2$	220		–	+
32.	44.49	Propanoic acid, 2-methyl-2[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{10}H_{24}O_3Si_2$	248		–	+
33.	47.34	Tris(trimethylsilyl)borate	$C_9H_{27}BO_3Si_3$	278		–	+
34.	49.61	1,3-Heptanol, trimethylsilyl	$C_{10}H_{24}OSi$	188		–	+
35.	51.54	2-Butene-1,4-diol, bis(trimethylsilyl)	$C_{10}H_{24}O_2Si_2$	232		–	+

Table 3. Phytotoxicity of tannery effluent before and after bacterial treatment.

TE (%)	GP (%)	SLM (%)	GI (%)	SL (cm)	RL (cm)	RSR	SVI	PP (%)	α-amylase activity (unit gr^{-1})
UT-TE (25 %)	90.00	10.00	47.04	2.74 ± 0.36	2.07 ± 0.10	0.75 ± 0.27	432.90	47.72	0.62 ± 0.04
BT-TE (25 %)	100.00	0.00	67.17	4.73 ± 0.41	2.66 ± 0.37	0.56 ± 0.90	739.00	32.82	0.65 ± 0.04
UT-TE (50 %)	70.00	30.00	23.33	2.15 ± 0.10	1.32 ± 0.47	0.61 ± 4.70	242.90	66.66	0.52 ± 0.04
BT-TE (50 %)	100.00	0.00	52.02	4.23 ± 0.20	2.06 ± 0.09	0.48 ± 0.45	629.00	47.97	0.60 ± 0.02
UT-TE(75 %)	53.33 ± 0.47	46.67	9.23	1.07 ± 0.56	0.69 ± 0.14	0.64 ± 0.25	93.86	82.57	0.41 ± 0.05
BT-TE(75 %)	90.00	10.00	37.72	3.33 ± 0.23	1.66 ± 0.23	0.49 ± 1.00	449.10	58.08	0.48 ± 0.03
UT-TE (100 %)	33.66 ± 0.47	66.34	2.75	0.50 ± 0.24	0.33 ± 0.12	0.66 ± 0.50	27.93	91.66	0.34 ± 0.02

BT-TE(100 %)	70.00	30.00	20.50	2.23 ± 0.20	1.16 ± 0.28	0.49 ± 1.40	244.30	70.70	0.42 ± 0.00
Control (TP)	100.00	0.00	100.00	5.80 ± 0.43	3.90 ± 0.29	0.68 ± 1.27	976.00	0.00	0.68 ± 0.01

TE TE $^{+}$

TE: Tannery effluent; UT-TE: Untreated tannery effluent; BT-TE: Bacterially (consortial) treated tannery effluent; TP: Tap water (control); GP: Germination percentage; SLM: Seedling mortality; GI: Germination index; SL: Shoot length; RL: Root length; RSR: Root-shoot ratio; SVI: Seed vigour index; PP: Phytotoxicity percentage.

All the values are mean of three replicates ± SD.

A high concentration of salts, noxious organic and inorganic contaminants (metals) has been reported to inhibit the phytohormones (like gibberellins, cytokinins, and auxins) that are crucial in the germination/ growth of seed/seedling [36]. The toxicity of untreated TE was also confirmed by seedling mortality (SLM) that was maximum (66.34 %) at a higher effluent concentration (100 %, v/v) as compared to control (TP, 0.00 %). However, the SLM was substantially decreased from higher to lower concentrations (100 % - 25 %, v/v) of TE after treatment with bacterial consortium GS-TE1310. The germination index (GI) in the seeds irrigated with tap-water (control) was found to be 100 % reported to vary from 47.04 % to 2.75 % from low to high concentrations (25 %– 100 %, v/v) of untreated TE; however, after bacterial treatment, it was significantly improved from higher to lower concentrations (100 % - 25 %, v/v) of treated TE. The seeds irrigated with control (TP) showed the normal growth of both root and shoot but, seeds irrigated with 100 % untreated TE exhibited a very small shoot (0.50 ± 0.24 cm) and root (0.33 ± 0.12 cm) development. The possible cause for the reduction of shoot and root growth were might be the presence of high salt concentration, COD, TSS, recalcitrant organic and inorganic chemicals in the untreated TE (Table 1). However, after treatment with bacterial consortium GS-TE1310, the shoot and root growth were significantly improved from higher to lower concentrations (100 % - 25 %, v/v) of treated TE. Similarly, the seeds treated with control (TP) showed a very high (976.00) seed vigour index (SVI) as compared to that of seeds irrigated with untreated TE. However, SVI was significantly improved in seeds that were irrigated with TE treated with bacterial consortium GS-TE1310.

Further, the activity of α-amylase enzyme noted in the germinating seeds evidently confirmed the toxic nature of untreated TE towards the germination/growth of seed/seedling (Table 3). A optimal activity of α-amylase (0.62 ± 0.04 Unit grain^{-1}) was noted in the seeds irrigated with 25 % concentration (v/v) of untreated TE and afterward, it was continuously decreased at sequentially higher volume (i.e. 50 %–75 %, v/v) of the TE. A concentration of salts, toxic heavy metals, and recalcitrant organic pollutants have been reported to inhibit the activity of α-amylase crucial for the splitting of starch into sugars and thus, it is useful in seed germination [29]. However, the activity of α-amylase was significantly improved in the seeds irrigated with higher to lower concentrations (100 % - 25 %, v/v) of TE treated with bacterial consortium GS-TE1310. The improved α-amylase activity in the seeds irrigated with treated TE was corroborated to the degradation/detoxification of organic chemicals and heavy metals in the untreated TE by bacterial consortium GS-TE1310 [29]. The results obtained at 25 and 50 % concentration (v/v) of bacterially treated TE were invariably better as compared to untreated leather TE. Further, these concentrations (25 and 50 %, v/v) of bacterially treated TE might be acted like a liquid fertilizer and non-toxic to plant growth and development [29], and hence, could be used to irrigate the agricultural crops with correct dilution.

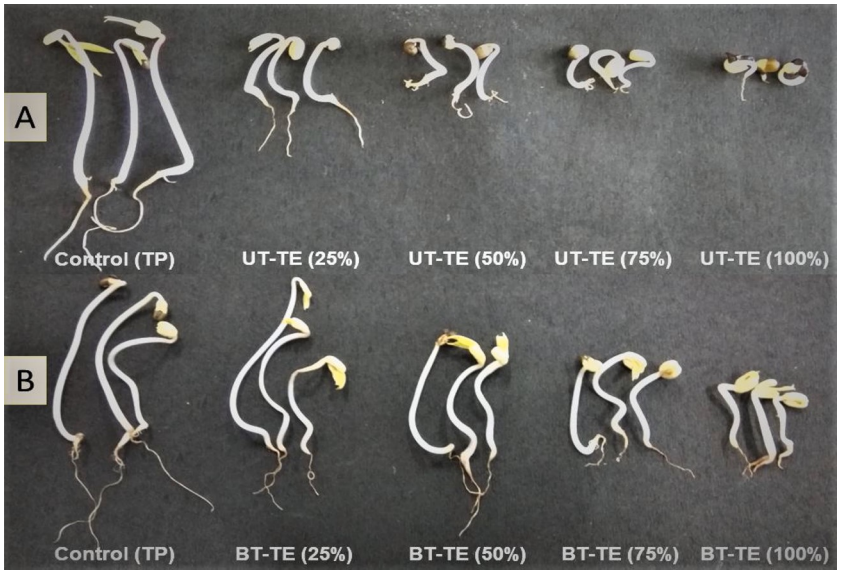


Fig. 7. Effect of (A) untreated (UT) and (B) bacterial treated (BT) tannery effluent (TE) at the concentration of 25 %, 50 %, 75 % and 100 % on seedling growth of Phaseolus aureus L.

4. Conclusions

The present study was aimed to develop a novel bacterial consortium GS-TE1310 for the degradation and detoxification of real leather TE and its phytotoxicity evaluation for environmental safety. In the present study, three potential pollutants degrading bacterial strains GS1, GS3, and GS10 were isolated from secondary treated leather TE + sludge, which were able to tolerate up to 6, 4, and 8% salt (NaCl) concentration and capable to remove COD up to 61.12, 54.28, and 66.32 % from real leather TE, respectively, and hence, used in the development of a new bacterial consortium GS-TE1310. Further, these bacterial strains were identified as *Ochrobactrum intermedium* GS1*, Micrococcus lylae* GS3, and *Stenotrophomonas acidaminiphila* GS10, respectively, based on various morphological and biochemical characterizations and 16S rRNA gene sequence analysis. The physico-chemical analysis of leather TE used in bioremediation studies revealed high COD, BOD, TDS, phenol and total Cr and also showed phytotoxic effects to *Phaseolus aureus* L, a terrestrial model organism. During bioremediation studies, the newly developed bacterial consortium GS-TE1310 effectively reduced all the pollution parameters (BOD: 85.32 %, COD: 76.12 %, TDS: 71.89 %, phenol: 88.70 %, and total chromium: 71.22 %) simultaneously from real leather TE within 120 h and thus, compellingly showed a significant potential for TE treatment and detoxification. The optimum pH, temperature, inoculum concentration, and agitation rate were found to be 7, 35 °C, 20 mL and 120 rpm, respectively and the best C and N-source was glucose and ammonium chloride amongst the different C and N-sources used for the bioremediation of leather TE. Further, organic contaminants identified in the untreated TE were completely removed in the bacterially treated TE and new metabolic products were formed as confirmed by FT-IR, HP- LC and GC–MS techniques. Phytotoxicity studies also unfolded that the toxicity of bacterially treated TE was reduced significantly allowing the 70 % germination of seeds as compared to seeds irrigated with untreated TE (100 %, v/v). Thus, the bacterially treated TE could be used as a liquid fertilizer to irrigate agricultural crops with suitable dilution. Overall, the present study suggests

that the newly developed bacterial consortium GS-TE1310 was more effective in the treatment/detoxification of leather TE for environmental protection.

CRediT authorship contribution statement

Gaurav Saxena: Conceptualization, Methodology, Validation, Data curation, Writing - review & editing, Visualization, Funding acquisition. **Diane Purchase:** Software, Writing - review & editing. **Sikandar I. Mulla:** Formal analysis, Writing - review & editing. **Ram Naresh Bharagava:** Conceptualization, Supervision, Resources, Project administration, Funding acquisition, Investigation.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The financial support received from the Science and Engineering Research Board (SERB), Department of Science & Technology (DST), Government of India (GOI), New Delhi, India as "Major Research Project" (Grant No.: EEQ/2017/000407) and University Grant Commission (UGC), Government of India (GOI), New Delhi, India is duly acknowledged. We thank Dr. Mukesh Kumar, University Science and Instrumentation Centre (USIC), Babasaheb Bhimrao Ambedkar University (BBAU), Lucknow for FT-IR analysis; Dr. D. K. Patel, Council of Scientific & Industrial Research (CSIR)-Indian Institute of Toxicology Research (IITR), Lucknow for HP-LC and GC–MS Analysis; and Dr. Subhash Awasthi, In-charge CETP, Unnao (India) for kindly providing the secondary treated TE samples for studies.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.jwpe.2020.101592.

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